Genetic Structure and Relationships among Populations of the Caspian Bent-toed Gecko, *Tenuidactylus caspius* (Eichwald, 1831) (Sauria: Gekkonidae) in Northern Iran

Vida HOJATI^{1*}, Eskandar Rastegar POUYANI² and Kazem PARIVAR³

Abstract The Caspian bent-toed gecko, *Tenuidactylus caspius*, belonging to the family Gekkonidae, is widely distributed across the northern half of Iran, especially along the southern coastal region of the Caspian Sea. It is regarded as a commensal species throughout its entire distribution. We investigated genetic variation and relationships among different populations of this species in Iran. Fragments of the mitochondrial cyt *b* (547 bp) and ND4 (831 bp) genes were sequenced and analyzed in 64 and 28 specimens, respectively, from 21 geographically distant localities. *Cyrtopodion scabrum* was used as the outgroup taxon. The data showed no significant genetic variation within the populations of *T. caspius* in Iran. Nevertheless, populations of Khorasan and Semnan (especially Shahrood) in northeastern Iran showed greater divergence (*p*-distance = 2.1%) from other Iranian populations. The low genetic variation and homogeneous structure among populations of *T. caspius* on either side of the Elburz Mountains suggests that this species most likely has achieved its current distribution recently and as a result of anthropogenic activities.

Keywords mtDNA, *Tenuidactylus caspius*, Gekkonidae, phylogeny, homogeneity, Iranian Plateau.

1. Introduction

The genus *Tenuidactylus* comprises seven species, three of which occur in Iran, chiefly in the eastern and northern portions of the country (Bauer *et al.*, 2013). *Tenuidactylus caspius* (Eichwald, 1831) is the most common gecko in northern Iran and comprises two subspecies in the Caspian Sea region (Leviton *et al.*, 1992; Szczerbak and Golubev, 1996; Anderson, 1999). *T. caspius caspius* is widely distributed in the eastern part of the Caucasus and Central Asia (Szczerbak, 2003; Kami, 2005). *T. caspius insularis* (Akhmedov and Szczerbak, 1978) occurs on the island of Vulf in the Caspian Sea and is known only from the type locality. It differs from the nominate subspecies

In Iran *Tenuidactylus caspius* occurs in the northern part of the Iranian Plateau, an area affected by geological events in the Miocene (7-15 Million years ago). In particular, the uplift of the Elburz Mountains as well as the Kopet Dagh and Caucasus regions has strongly influenced the diversity and evolution of reptiles in the area (Rastegar-Pouyani *et al.*, 2012). Leviton and Anderson (1984) indicated that divergence in the genus *Tenuidactylus* had started in the early Miocene (16-23 Millions of years ago, Ma). Bauer *et al.* (2013) suggested that the split between *T. caspius* plus *T. fedtschenkoi* and the other species of the genus had occurred 12 (7-17) Ma as a vicariant speciation event due to orogenic

Received:7 March 2014 Accepted: 11 May 2015

¹ Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran

² Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran

³ Department of Biology, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran

in having the first pair of postmental shields usually separated from each other by gular scales, although they may contact one another at a point, whereas in the nominate subspecies they are in broad contact (Akhmedov and Szczerbak, 1978).

^{*}Corresponding author: Dr. Vida HOJATI, from Developmental Biology, Islamic Azad University, Iran, with her research focusing on reptiles. E-mail: vida.hojati@gmail.com

events in Iran and the Transcaspian region. The process of diversification in *Tenuidactylus* may be comparable to that in the genus *Teratoscincus*, which had also been diversified in Central Asia (Macey *et al.*, 2005). Nonmonophyly of the genus *Cyrtopodion senso lato* has been demonstrated in several studies (Macey *et al.*, 2000; Gamble *et al.*, 2012) and the genus *Tenuidactylus* is considered as sister to a larg clade including, *Agamura*, *Crossobamon*, *Bunopus* and *Cyrtopodion* (Bauer *et al.*, 2013).

Ahmadzadeh *et al.* (2010) examined morphological variation among populations of *T. caspius* in Iran, especially between the Moghan and Damghan populations. They clarified the presence of interpopulational variation in most characters with particular emphasis of larger body size in Moghan specimens than in Damghan specimens. In a more recent study, however, no morphological differences (metric or meristic) were found among populations of this species from across all parts of northern Iran (Hojati, 2012).

Intraspecific differentiation of a species is usually attributed to geographic, demographic, and ecological factors that have operated throughout its evolutionary history (Walker and Avise, 1998). This may be particularly apparent in taxa that show only limited mobility, such as reptiles, while commensal geckos can easily be transported into new regions by anthropogenic means (e.g., *Hemidactylus turcicus* in the USA: Davis,

1974; Kraus, 2009). Molecular markers are of great value to study intraspecific variation and its geographic association, and to infer the evolutionary history of a species, especially in cases of phenotypic variation (Moritz and Hillis, 1996; Cruzan and Templeton, 2000).

We therefore addressed the question of intraspecific differentiation in *T. caspius* by inferring a molecular phylogeny using mitochondrial cytochrome b (cyt *b*) and ND4 gene sequences. These markers have proven to be very useful in various investigations of molecular phylogeography and systematics in reptiles (e.g., Wink *et al.*, 2001; Guicking *et al.*, 2002a, b; Nagy *et al.*, 2002; Carranza *et al.*, 2004; Carranza and Arnold, 2006; Carranza and Arnold, 2012; Kindler *et al.*, 2013).

2. Materials and Methods

2.1 Sampling A total of 64 specimens of *Tenuidactylus caspius* were collected between 2011 and 2012 from 21 geographically distant localities covering all parts of the species' distributional range in Iran (Figure 1). Tissue samples were preserved in absolute ethanol and were deposited in the Sabzevar University Herpetological Collection (SHUC). Based on the present knowledge of the phylogenetic relationships among *Tenuidactylus* and its allied genera, *Cyrtopodion scabrum* was chosen as an outgroup taxon. The complete list of materials examined and their GenBank accession numbers are given in Appendix 1 and 2.

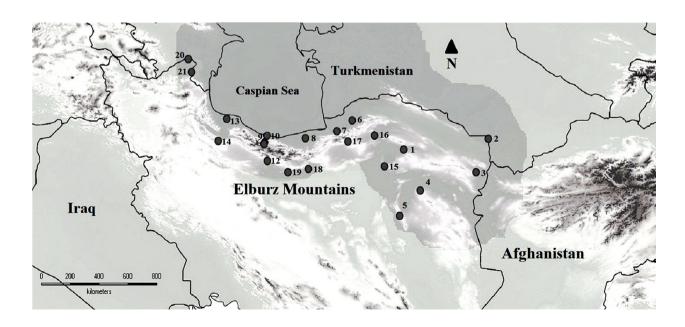


Figure 1 Map of Iran and adjacent countries, showing distribution of *Tenuidactylus caspius* and localities in which samples for this study were collected. Numbers in blue circles indicate the location number.

2.2 DNA extraction and PCR amplification Genomic DNA from each muscle and liver tissue sample was extracted using the salt method (Kabir *et al.*, 2006). Fragments of the mitochondrial cytochrome b (cyt *b*) and NADH Dehydrogenase subunit 4 (ND4) genes were amplified with the primers Mta_new and Ei700r (Rastegar-Pouyani *et al.*, 2010) and ND4 and Leu (Arévalo *et al.*, 1994), respectively. Relevant programs for PCR were extracted from following references (Arévalo *et al.*, 1994; Rastegar-Pouyani *et al.*, 2010) and were slightly modified for use in *Tenuidactylus*.

2.3 Phylogenetic analyses DNA sequences of 547 bp of cyt *b* and 831 bp of ND4 were aligned using BioEdit 7.0 (Hall, 1999) with default parameters. As both of the genes sequenced are protein coding, nucleotide sequences revealed were translated into amino acid sequences to evaluate the presence of inspected stop codons (none were detected). To perform ML and Bayesian analyses, the best-fitting evolutionary model was chosen for our dataset using jModelTest 2.1.1 (Posada, 2008), under corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC).

Three methods of phylogenetic analyses were performed: Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI). Based on the present knowledge of phylogenetic relationships among gekkotan lizards, sequences of Cyrtopodion scabrum, retrieved from the GenBank, were selected as the outgroup taxon. ML was conducted using the selected 27 sequences as a combined data set of cyt b and ND4 with the program RaxML ver. 7.0.3 (Stamatakis, 2006) under GTRGAMMA model with 1000 bootstrap replicates. In this analysis, Tropiocolotes steudneri was chosen as the outgrop taxon due to its close affinity to Tenuidactylus (Bauer et al., 2013), and GenBank sequence availability for both genes of interest. Maximum Parsimony analyses were performed in PAUP*4.0 (Swofford, 2003) with all sites weighted equally; saturation effects were negligible in our data set. A Bayesian analysis was carried out using Mr.Bayes 3.1.2 (Huelsenbeck and Ronquist, 2001). A partitioned Bayesian analysis was performed in four chains and two independent runs for four million generations with model parameters for each gene partition (GTR + I + G for both cyt b and ND4). This model was obtained using the program iModelTest with AICc criterion. The analyses were started with randomly generated trees and every 100th tree was sampled. The log-likelihood of the 100000 trees in each analysis was plotted against the generation time. After verifying that saturation had been reached,

both in the term of likelihood scores and parameter estimation, the first 25% of trees were discarded in both runs, and a majority-rule consensus tree was generated from the remaining 75% (postburnin) trees. The frequency of any particular clade among the individual trees contributing to the consensus tree represents the posterior probability of that clade (Huelsenbeck and Ronquist, 2001). Average uncorrected genetic distances (p-distance) between groups of T. caspius were calculated in MEGA 5.1 (Tamura et al., 2011). Combined sequences of cyt b and ND4 for 27 samples were employed to create haplotype network. For this purpose, sequences inserted to the software PHASE v. 2.1.1 that implemented in DNAsp (Librado and Rozas, 2009) for resolving phased haplotypes (Stephens et al., 2001). Haplotype network were done using Network v. 4.5.1.0 (Bandelt et al., 1999) with median joining option and default setting.

3. Results

Our Tenuidactylus caspius dataset of aligned sequences consisted of 547 bp of cytochrome b for 64 specimens and 831 bp of ND4 for 28 individuals. The best fit model of sequence evolution was GTR+G+I for cyt b; GTR+G for ND4 and HKY +G model for the combined dataset. Results from the three methods of phylogenetic analyses, to the great extant, supported the similar tree topology for both genes, either for individual gene trees or in the trees recovered from the combined dataset. BI tree for cyt b and ML tree for the combined dataset are presented in Figures 2 and 3, respectively. Due to extensive non-overlap of samples for the two genes, we did not concatenate our data for all samples included in the study. Two major clades can be defined within the phylogenetic trees, North/northwest and East/northeast clades. Clade separation is relatively well supported (91% of bootstrap value) in the cyt b tree. In addition, within the East/ northeast clade, populations of Khorasan Province (Sabzevar, Bejestan, Sarakhs, Torbat Jam, boshrouyeh and Gonabad) and Semnan (especially Shahrood) populations were clearly differentiated from each other. The North/northwest clade is a quite heterogeneous clade with several subclades, however the amounts of genetic divergence among the subclades are relatively small. In general, all inter-population divergences within the local populations of this species in Iran are small (Table 1); 1.2% to 2% among the clades. Haplotype network were provided for 27 combined sequences of cyt b and ND4 (Figure 4) and as it is clear, northern and northwestern populations are divided from Semnan and eastern

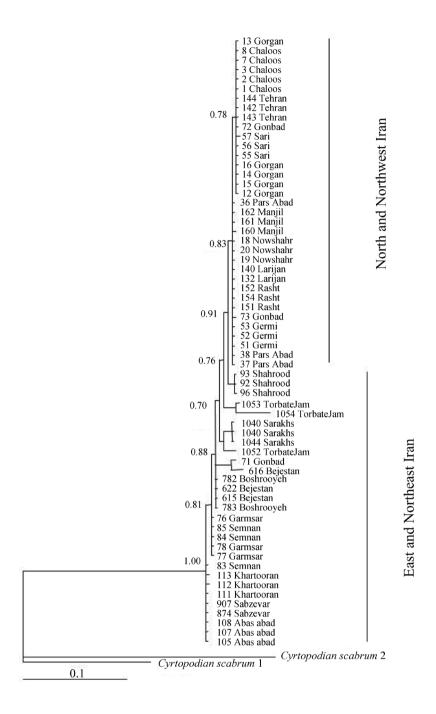


Figure 2 Phylogenetic relationships among the *T. caspius* populations included in the analysis. Individuals of *C. scabrum* were used as outgroups. Only the BI tree of cyt *b* is presented. Numbers close to the branches are posterior probabilities.

populations (Khorasan). Haplotype diversity estimated as 0.9516. Having 16 different haplotypes within the vast range of this species in Iran possibly indicates a relatively recent dispersal and diversification of the clade in the area a whole.

The highest genetic diversity (*p*-distance) of cyt *b* between populations was 2%, between the Shahrood (clade 4) and Khorasan populations (clade 5) (Figure 2).

The lower divergence value was 1.2% between North + Tehran and Shahrood populations (clades 2 and 4, respectively).

As shown in Table 1, the *T. caspius* populations, based on sequencing of cyt *b* gene, have much larger cyt *b p*-distances of 23.5% to 24.6% with outgroup taxon.

Genetic distances for ND4 sequences (28 specimens) show a high similarity between Khartooran and Semnan

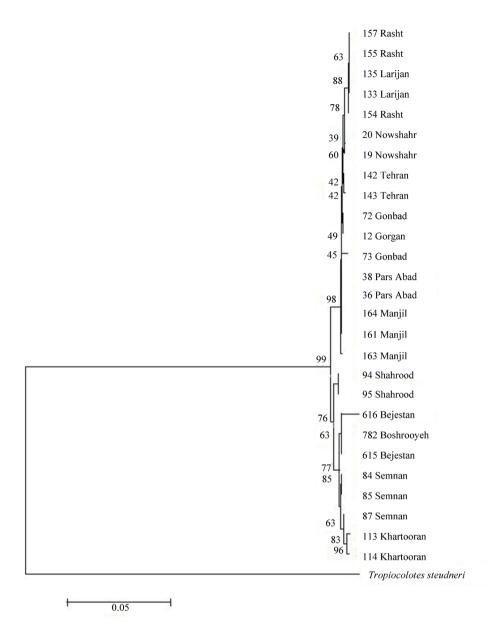


Figure 3 ML analysis of the phylogenetic relationships among populations of *T. caspius* based on 1378 bp of cyt *b* and ND4 sequences. Numbers close to the nodes are bootstrap supports with 2000 replicates.

Table 1 Genetic distances (*p*-distance) between major clades of the *T. caspius* complex included in this study (Cyt *b*).

	[1]	[2]	[3]	[4]	[5]
[1]					
[2]	0.244				
[3]	0.235	0.013			
[4]	0.246	0.012	0.015		
[5]	0.242	0.019	0.016	0.02	

[1] = Outgroup, [2] = North + Tehran, [3] = Semnan+Khartooran, [4] = Shahrood, [5] = Khorasan.

populations and a divergence of approximately 2% from all other clades. Both markers suggest that the species does not exhibit clear divergence among its Iranian populations; rather there is only a low variation (2%) between eastern and north-northwestern clades.

4. Discussion

Geographic genetic variation among conspecific populations is usually affected by both ecological and natural factors. Species adaptation and tolerance in different habitats are highly related to the ecological

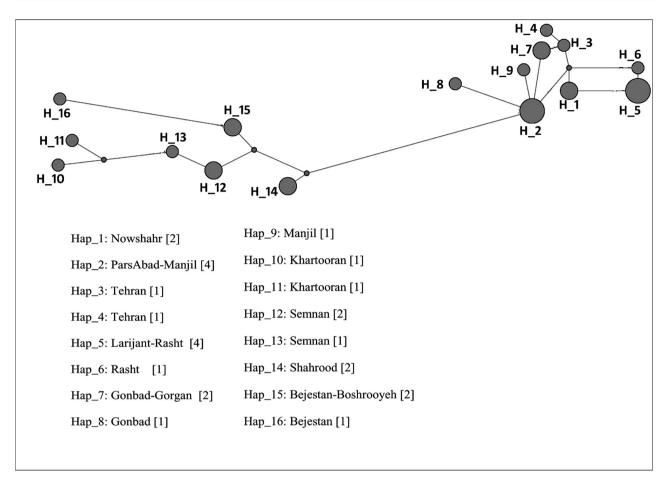


Figure 4 Parsimony network related to cyt *b* and ND4 sequences. Lines represent a mutational step, circles haplotypes and dots unsampled haplotypes. The size of circles is proportional to the number of individuals. Numbers next to the circles represent the haplotype localities as shown in the bottom of figure separately.

factors of the habitats that species can dwell but natural factors are related to population's dynamics and dispersal waves and vicariance events (Riddly, 2004). Samples used in this study were collected from throughout the area of the species' distribution in Iran (Figure 1) and neither of the mitochondrial genes showed significant differences among populations, indicating a homogeneous genetic structure within the species populations in a vast area of distribution rang in Iran. There are several polytomies in the both individual and combined trees, (Figures 2 and 3) this also in turn indicates that either the populations of T. caspius are still not genetically diverged properly or markers used in the study are not sufficient enough to reveal real divergence among the clades. However, a massive body of recent molecular studies indicated that the markers used in this study are quite adequate for revealing genetic divergence among populations of a species (e.g., Heidari et al., 2011). A comparable study on another widespread gecko in Iran, Cyrtopodion scabrum, using sequences of cyt b shows the similar results (Fili,

2012).

Several comprehensive studies have been done to estimate the phylogenetic relationships among geckos. Nazarov and Poyarkov (2013) performed a project on the genus *Cyrtopodin* using a mitochondrial gene (COI), which resulted in recognition of an undescribed species of this genus in Uzbekistan.

Based on previous molecular studies, *T. caspius* was identified as an extant but old species (Bauer *et al.*, 2013). This species is estimated to have diverged from the closely related *T. fedtschenkoi* about 12 Ma when the Kopet Dagh was not yet raised and the Elburz Mountains had started to uplift (Alaeei, 2009).

Hojati (2012) studied *Tenuidactylus caspius* morphologically and found no significant differences across northern Iran. In contrast, however, Ahmadzadeh *et al.* (2010) argued that this species is morphologically relatively variable in Iran. On the other hand, Oraei (2009) and Fili (2012) showed high genetic and morphologic homogeneity in all populations of

Cyrtopodion scabrum in Iran and Hojati (2012) also presented a similar result for *T. caspius*. The main finding of this study is that despite its vast distribution area with many local populations, *T. caspius* is a homogeneous clade and there are no significant genetic differences among geographically distant populations.

Genetic variation among the Iranian populations of *Tenuidactylus caspius* is low and the genetic structure of this species is relatively homogeneous. Our data show that despite the presence of a vast geographic barrier (the Elburz Montains), the genetic distance between Tehran and North Iran populations (12 for Tehran and 9-10-11 for north group in Figure 1) are very low. It appears that anthropogenic activities may be responsible for the recent dispersal of this species in the area as a similar situation has been documented for *Cyrtopodion scabrum* in Iran (Fili, 2012). *T. caspius* can be found around humans and is known as a commensal species in northern Iran. Such movements by humans swamp out the effects of natural barriers and historical biogeography on patterns of variation.

Acknowledgements We are thankful to the authorities at Hakim Sabzevari University, Iran, for providing the Lab. work facilities. Special thanks go to Reza BABAEI SAVASARI and Afshin FAGHIRI for their unforgettable help in collecting samples at night. We are also grateful of Prof. Aaron BAUER from Villanova University who commented on an earlier draft of the manuscript and edited our English. The work was partially supported by the Iran National Scientific Foundation (INSF) under proposal number of 89001493.

References

- **Ahmadzadeh F., Hojati V., Faghiri A.** 2010. Morphological variation between three populations of the Caspian Bent-toed gecko, *Cyrtopodion caspium* (Eichwald, 1831) in northern Iran: evidence for incipient speciation? Zool Middle East, 51: 31–38
- Akhmedov M. I., Szczerbak N. N. 1978. *Gymnodactylus caspius insularis* ssp. n. (Reptilia, Sauria), a new subspecies of *Gymnodactylus caspius* EICHW. from Vulf Island in the Caspian Sea. Vestnik Zoologii (Kiev), 2: 80–82 (in Russian)
- **Alaeei M.** 2009. Geomorphology of Iran. Ghoomes Publication Company, Tehran, Iran. 360 Pp.
- Anderson S. C. 1999. The Lizards of Iran. Society for the Study of Amphibians and Reptiles, Ithaca, NY, pp 442
- Arévalo E., Davis S. K., Sites J. W. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst Biol, 43: 387–418
- **Bandelt H. J., Forster P., Rhl A.** 1999. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol, 16: 37–48

- Bauer A. M., Masroor R., Titus-Mcquillan J., Heinicke M. P., Daza J. D., Todd R. 2013. A preliminary phylogeny of the Palearctic naked-toed geckos (Reptilia: Squamata: Gekkonidae) with taxonomic implications. Zootaxa, 3599 (4): 301–324
- Carranza S., Arnold E. N., Amat F. 2004. DNA phylogeny of Lacerta (Iberolacerta) and other lacertinae lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? Syst Biodivers, 2: 57–77
- **Carranza S., Arnold E. N.** 2006. Systematics, biogeography and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. Mol Phylogenet Evol, 38: 531–545
- Carranza S., Arnold E. N. 2012. A review of the geckos of the genus *Hemidactylus* (Squamata: Gekkonidae) from Oman based on morphology, mitochondrial and nuclear data, with descriptions of eight new species. Zootaxa, 3378: 1–95
- Cruzan M. B., Templeton A. R. 2000. Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. Trends Ecol Evol, 15: 491–496
- **Davis W. K.** 1974. The Mediterranean Gecko, *Hemidactylus turcicus* in Texas. J Herpetol, 8: 77–80
- Fili N. 2012. The study of interspecies variations in different populations of *Cyrtopodion scabrum* in Iran. MSc. Thesis in Animal Biosystematics, Lorestan University, Iran
- Gamble T., Greenbaum E., Jackman T. R., Russell A. P., Bauer A. M. 2012. Repeated Origin and Loss of Adhesive Toe pads in Geckos. PLoS ONE, 7(6): e39429. doi:10.1371/journal.pone. 0039429
- Guicking D., Fritz U., Wink M., Lehr E. 2002a. New data on the diversity of the Southeast Asian leaf turtle genus Cyclemys Bell, 1834. Molecular results (Reptilia: Testudines: Geoemydinae). Faunistische Abhandlungen Staatliches Museum für Tierkunde Dresden, 23: 75–86
- **Guicking D., Joger U., Wink M.** 2002b. Molecular phylogeography of the viperine snake (*Natrix maura*) and the Dice snake (*Natrix tessellata*): first results. Biota, 3: 49–5
- **Hall T. A.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser, 41: 95–98.
- Heidari N., Rastegar-Pouyani E., Rastegar-Pouyani N., Faizi H. 2014. Molecular phylogeny and biogeography of the genus Acanthodactylus Fitzinger, 1834 (Reptilia: Lacertidae) in Iran, inferred from mtDNA Sequences. Zootaxa, 3860(4): 379–395
- Hojati V. 2012. Molecular phylogeny and gametogenesis study on the Caspian bent-toed gecko, *Cyrtopodion caspium* (Sauria: Gekkonidae) in northern Iran. Ph.D Thesis in Developmental Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran, pp 217
- **Huelsenbeck J. P., Ronquist F.** 2001. Mr. Bayes: Bayesian inference of phylogeny. Bioinformatics, 17: 754–755
- Kabir S., Shahriar M., Hamidul Kabir A. N. M., Uddin M. G. 2006. High salt SDS-based method for the direct extraction of genomic DNA from three different gram-negative organisms. The CDR Journal, 1: 57–64
- **Kami H. G.** 2005. *Cyrtopodion caspium caspium* (Caspian benttoed gecko, or Caspian thin-toed gecko. Herpetol Rev, 36: 79
- Kindler C., Bhme W., Corti C., Gvod&k V., Jablonski D., Jandzik D., Metallinou M., Cirok P., Fritz U. 2013.

- Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix*, *N. megalocephala*). Zool Scr, 42: 458–472
- **Kraus F.** 2009. Alien Reptiles and Amphibians: a Scientific Compendium and Analysis. Springer Verlag, Dordrecht, The Netherlands, pp xii + 564 pp
- **Leviton A. E., Anderson S. C.** 1984. Description of a new species of *Cyrtodactylus* from Afghanistan with remarks on the status of *Gymnodactylus longipes* and *Cyrtodactylus fedtschenkoi*. J Herpetol, 18: 270–276
- Leviton A. E., Anderson S. C., Adler K. K., Minton S. A. 1992.
 Handbook to Middle East Amphibian and Reptiles. Contribution to Herpetology No: 8. Society for the Study of Amphibians and Reptiles. Oxford, OH. pp vii + 252 pp
- **Librado P., Rozas J.** 2009. DnaSP v5: software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451–1452
- Macey J. R., Ananjeva N. B., Wang Y., Papenfuss T. J. 2000. Phylogenetic relationships among Asian gekkonid lizards formerly of the genus *Cyrtodactylus* based on cladistic analyses of allozymic data: monophyly of *Cyrtopodion* and *Mediodactylus*. J Herpetol, 34: 258–265
- Macey R., Fong J. J., Kuehl J. V., Shafei S., Ananjeva N. B., Papenfuss T. J., Boore J. L. 2005. The complete mitochondrial genome of a gecko and the phylogenetic position of the Middle Eastern *Teratoscincus keyserlingii*. Mol Phylogenet Evol, 36: 188–193
- Moritz C., Hillis D. M. 1996. Molecular systematics: context and controversies. In: Hillis DM, Moritz C, Mable BK (eds), Molecular Systematics. Sinauer Associates, Sunderland, MA, pp 1–113
- Nagy Z. T., Joger U., Guicking D., Wink M. 2002. Phylogeography of the European Whip snake *Coluber* (*Hierophis*) *viridiflavus* as inferred from nucleotide sequences of the mitochondrial cyt b gene and ISSR genomic fingerprinting. Biota, 3: 109–118
- Nazarov R. A., Poyarkov N. A. 2013. A Taxonomic revision of the genus *Tenuidactylus* Szczerbak le Golubev 1984 (Reptilia: Squamata: Gekkonidae) with a description of a new species from Central Asia. Zool J, 92(11): 1312–1332 (in Russian)
- **Oraci H.** 2009. Biosystematics and biogeography of Gekkonidae family with on *Cyrtopodion* genus in Iran. MSc. Thesis in Animal Biosystematics, Razi University, Kermanshah, Iran.

- **Posada, D.** 2008. jModelTest: phylogenetic model averaging. Mol Biol Evol, 25(7): 1253–1256
- Rastegar-Pouyani E., Kazemi Noureini S., Rastegar-Pouyani N., Joger U., Wink M. 2010. Molecular phylogeny and biogeography of the *Eremias persica* complex of the Iranian Plateau (Reptilia: Lacertidae) based on sequences of the mtDNA. Zool J Linn Soc, 158: 641–660
- Rastegar-Pouyani E., Kazemi Noureini S., Rastegar-Pouyani N., Joger U., Wink M. 2012. Molecular phylogeny and intraspecific differentiation of the *Eremias velox* complex of the Iranian Plateau and Central Asia (Sauria, Lacertidae). J Zool Syst Evol Res, 50: 220–229
- Riddly M. 2004. Evolution. 3rd Edition Blackwell Publishing, Malden, 751 pp-
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22: 2688–2690
- Stephens M., Smith N. J., Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. Am J Hum Genetics, 68: 978–989
- **Swofford D. L.** 2003. PAUP*. Phylogenetic analysis using parsimony (* and other methods), Version 4.0b10. Massachusetts: Sinauer Associates, Sunderland.
- Szczerbak N. N., Golubev M. L. 1996. The gecko fauna of the USSR and adjacent regions [English ed., translated from the Russian by ML Golubev & SA Malinsky; AE Leviton & GR Zug, eds.]. Society for the Study of Amphibians and Reptiles, Ithaca (New York), 232 pp., 8 pls.
- **Szczerbak N. N.** 2003. Guides to the Reptiles of the Eastern Palaearctic. Krieger Publishing Company, Malabar, FL.
- Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA 5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol, 28: 2731–2739
- Walker D., Avise J. C. 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Ann Rev Ecol Sys, 29: 23–58
- Wink M., Guicking D., Fritz U. 2001. Molecular evidence for hybrid origin of Mauremys iversoni Pritchard et McCord, 1991, and Mauremys pritchardi McCord, 1997 (Reptilia: Testudines: Bataguridae). Zoologische Abhandlungen Staatliches Museum für Tierkunde Dresden, 51: 41–50

Appendix 1 List of samples of Tenuidactylus caspius with their geographical origin and relevant accession number in GeneBank.

Museum number	Species	Locality; number in Figure 1	Latitude (N) °	Longitude (E) °	Accession number
874	Tenuidactylus caspius	Sabzevar-1	31.12	57.43	KJ486228
907	Tenuidactylus caspius	Sabzevar-1	31.12	57.43	KJ486229
1040		Sarakhs-2	36.53	61.16	KJ486225
	Tenuidactylus caspius				
1039	Tenuidactylus caspius	Sarakhs-2	36.53	61.16	KJ486226
1044	Tenuidactylus caspius	Sarakhs-2	36.53	61.16	KJ486227
1052	Tenuidactylus caspius	Torbate Jam-3	35.24	60.62	KJ486222
1053	Tenuidactylus caspius	Torbate Jam-3	35.24	60.62	KJ486223
1054	Tenuidactylus caspius	Torbate Jam-3	35.24	60.62	KJ486224
615	Tenuidactylus caspius	Bejestan-4	34.51	58.17	KJ486232
616	Tenuidactylus caspius	Bejestan-4	34.51	58.17	KJ486233
622	Tenuidactylus caspius	Bejestan-4	34.51	58.17	KJ486234
782	Tenuidactylus caspius	Boshrooyeh-5	33.52	57.27	KJ486230
783	Tenuidactylus caspius	Boshrooyeh-5	33.52	57.27	KJ486231
71	Tenuidactylus caspius	Gonbad-6	37.25	55.17	KJ486193
72	Tenuidactylus caspius	Gonbad-6	37.25	55.17	KJ486194
73	Tenuidactylus caspius	Gonbad-6	37.25	55.17	KJ486195
13	Tenuidactylus caspius	Gorgan-7	36.83	54.48	KJ486179
12	Tenuidactylus caspius	Gorgan-7	36.83	54.48	KJ486180
15	Tenuidactylus caspius	Gorgan-7	36.83	54.48	KJ486181
14	Tenuidactylus caspius	Gorgan-7	36.83	54.48	KJ486182
16	Tenuidactylus caspius	Gorgan-7	36.83	54.48	KJ486183
55	Tenuidactylus caspius	Sari-8	36.55	53.1	KJ486190
56	Tenuidactylus caspius	Sari-8	36.55	53.1	KJ486191
57	Tenuidactylus caspius	Sari-8	36.55	53.1	KJ486192
19	Tenuidactylus caspius	Nowshahr-9	36.39	51.29	KJ486216
20	Tenuidactylus caspius	Nowshahr-9	36.39	51.29	KJ486217
18	Tenuidactylus caspius	Nowshahr-9	36.39	51.29	KJ486218
1	Tenuidactylus caspius	Chaloos-10	36.66	51.41	KJ486235
2	Tenuidactylus caspius	Chaloos-10	36.66	51.41	KJ486236
3	Tenuidactylus caspius	Chaloos-10	36.66	51.41	KJ486237
7	Tenuidactylus caspius	Chaloos-10	36.66	51.41	KJ486238
8	Tenuidactylus caspius	Chaloos-10	36.66	51.41	KJ486239
132	Tenuidactylus caspius	Larijan-11	36 06	52 15	KJ486214
140	Tenuidactylus caspius	Larijan-11	36 06	52 15	KJ486215
143	Tenuidactylus caspius	Tehran-12	35.67	51.42	KJ486208
142	Tenuidactylus caspius	Tehran-12	35.67	51.42	KJ486209
144	Tenuidactylus caspius	Tehran-12	35.67	51.42	KJ486210
151	Tenuidactylus caspius	Rasht-13	37.3	49.63	KJ486211
154	Tenuidactylus caspius	Rasht-13	37.3	49.63	KJ486212
152	Tenuidactylus caspius	Rasht-13	37.3	49.63	KJ486213
160	Tenuidactylus caspius	Manjil-14	36.44	49.25	KJ486219
161	Tenuidactylus caspius	Manjil-14	36.44	49.25	KJ486220
162	Tenuidactylus caspius	Manjil-14	36.44	49.25	KJ486221
111	Tenuidactylus caspius	Khartooran-15	35.47	56.6	KJ486240
112	Tenuidactylus caspius	Khartooran-15	35.47	56.6	KJ486241
113	Tenuidactylus caspius	Khartooran-15	35.47	56.6	KJ486242
105	Tenuidactylus caspius	Abbas Abad-16	36.33	51.28	KJ486205
107	Tenuidactylus caspius	Abbas Abad-16	36.33	51.28	KJ486206
108	Tenuidactylus caspius	Abbas Abad-16	36.33	51.28	KJ486207
93	Tenuidactylus caspius	Shahrood-17	36.42	54.97	KJ486202
96	Tenuidactylus caspius	Shahrood-17	36.42	54.97	KJ486203
92	Tenuidactylus caspius	Shahrood-17	36.42	54.97	KJ486204
83	Tenuidactylus caspius	Semnan-18	35.35	53.23	KJ486199
84	Tenuidactylus caspius	Semnan-18	35.35	53.23	KJ486200
85	Tenuidactylus caspius	Semnan-18	35.35	53.23	KJ486201
76	Tenuidactylus caspius	Garmsar-19	35.22	52.33	KJ486196
77	Tenuidactylus caspius	Garmsar-19	35.22 35.22	52.33	KJ486197
78	Tenuidactylus caspius	Garmsar-19	35.22	52.33	KJ486198
36	Tenuidactylus caspius	Pars Abad-20	39.65	47.93	KJ486184
37	Tenuidactylus caspius	Pars Abad-20	39.65	47.93	KJ486185
38	Tenuidactylus caspius	Pars Abad-20	39.65	47.93	KJ486186
51	Tenuidactylus caspius	Germy-21	39.13	48.08	KJ486187
52	Tenuidactylus caspius	Germy-21	39.13	48.08	KJ486188
53	Tenuidactylus caspius	Germy-21	39.13	48.08	KJ486189

Appendix 2 ND4 accession numbers (28 samples).

species	locality	Latitude (N) °	Longitude (E) °	Accession Number
Tenuidactylus caspius	20-Nowshahr	36.39	51.29	KJ486243
Tenuidactylus caspius	19-Nowshahr	36.39	51.29	KJ486244
Tenuidactylus caspius	25-Amol	36.23	52.20	KJ486245
Tenuidactylus caspius	28-Amol	36.23	52.20	KJ486246
Tenuidactylus caspius	38-Pars Abad	39.65	47.93	KJ486247
Tenuidactylus caspius	36-Pars Abad	39.65	47.93	KJ486248
Tenuidactylus caspius	122-Galogah	36.82	53.87	KJ486249
Tenuidactylus caspius	142-Tehran	35.67	51.42	KJ486250
Tenuidactylus caspius	143-Tehran	35.67	51.42	KJ486251
Tenuidactylus caspius	133-Larijan	36 06	52 15	KJ486252
Tenuidactylus caspius	135-Larijan	36 06	52 15	KJ486253
Tenuidactylus caspius	157-Rasht	37.30	49.63	KJ486254
Tenuidactylus caspius	155-Rasht	37.30	49.63	KJ486255
Tenuidactylus caspius	154-Rasht	37.30	49.63	KJ486256
Tenuidactylus caspius	163-Manjil	36.44	49.25	KJ486257
Tenuidactylus caspius	164-Manjil	36.44	49.25	KJ486258
Tenuidactylus caspius	161-Manjil	36.44	49.25	KJ486259
Tenuidactylus caspius	12-Gorgan	36.83	54.48	KJ486260
Tenuidactylus caspius	113-Khartooran	35.47	56.60	KJ486261
Tenuidactylus caspius	114-Khartooran	35.47	56.60	KJ486262
Tenuidactylus caspius	84-Semnan	35.35	53.23	KJ486263
Tenuidactylus caspius	85-Semnan	35.35	53.23	KJ486264
Tenuidactylus caspius	87-Semnan	35.35	53.23	KJ486265
Tenuidactylus caspius	94-Shahrood	36.42	54.97	KJ486266
Tenuidactylus caspius	95-Shahrood	36.42	54.97	KJ486267
Tenuidactylus caspius	615-Bejestan	34.51	58.17	KJ486268
Tenuidactylus caspius	616-Bejestan	34.51	58.17	KJ486269
Tenuidactylus caspius	782-Boshrooyeh	33.52	57.27	KJ486270